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Title	Role of Ca2+ stores in metabotropic L-glutamate receptor-mediated supralinear Ca2+ signaling in rat hippocampal neurons
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Publication date	2000-12-01
Original Citation	Rae, M.G., Martin, D.J., Collingridge, G.L. and Irving, A.J. (2000) 'Role of ca 2+ stores in metabotropicl-glutamate receptor-mediated supralinear ca 2+ signaling in rat hippocampal neurons', The Journal of Neuroscience, 20(23), pp. 8628–8636. https://doi.org/10.1523/JNEUROSCI.20-23-08628.2000
Type of publication	Article (peer-reviewed);Article
Link to publisher's version	10.1523/JNEUROSCI.20-23-08628.2000
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Role of Ca^{2+} Stores in Metabotropic L-Glutamate Receptor-Mediated Supralinear Ca^{2+} Signaling in Rat Hippocampal Neurons

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The role of metabotropic L-glutamate (mGlu) receptors in supralinear Ca^{2+} signaling was investigated in cultured hippocampal cells using Ca^{2+} imaging techniques and whole-cell voltage-clamp recording. In neurons, but not glia, global supralinear Ca^{2+} release from intracellular stores was observed when the mGlu receptor agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG) was combined with elevated extracellular K^+ levels (10.8 mM), moderate depolarization (15–30 mV), or NMDA (3 μM). There was a delay (2–8 min) before the stores were fully charged, and the enhancement persisted for a short period (up to 10 min) after removal of the store-loading stimulus. Studies with the mGlu receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine demonstrated that these effects were mediated by activation of the $mGlu_5$ receptor subtype. The L-type voltage-gated Ca^{2+} channel antagonist nifedipine (10 μM) substantially reduced responses to DHPG obtained in the presence of elevated extracellular K^+ but not NMDA. This suggests that the Ca^{2+} that is required to load the stores can enter either through L-type voltage-gated Ca^{2+} channels or directly through NMDA receptors. The findings that both depolarization and NMDA receptor activation can facilitate mGlu receptor Ca^{2+} signaling adds considerable flexibility to the processes that underlie activity-dependent changes in synaptic strength. In particular, a temporal separation between the store-loading stimulus and the activation of mGlu receptors could be used as a recency detector in neurons.

Key words: mGlu; NMDA; Ca^{2+} stores; Ca^{2+} release; recency detector; supralinear

Local and global elevations in neuronal cytosolic Ca^{2+} are important for a variety of physiological and pathological processes, including synaptic plasticity and gene expression (Berridge, 1998). The role of NMDA receptors in hippocampal Ca^{2+} signaling has been the subject of intense investigation (Mayer et al., 1987; Regehr and Tank, 1992; Segal and Manor, 1992; Alford et al., 1993; Perkel et al., 1993; Malinow et al., 1994; Petrozzino et al., 1995; Schiller et al., 1998; Emptage et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000), fueled by the role of these receptors in processes such as long-term potentiation (LTP) and long-term depression (LTD) (Bliss and Collingridge, 1993; Bear and Abraham, 1996). Of particular significance for hebbian plasticity, the biophysical properties of NMDA receptors enable them to act as coincidence detectors, whereby they only generate an effective response in the presence of a postsynaptic depolarization (Nowak et al., 1984; Mayer et al., 1984). These characteristics, coupled with their permeability to Ca^{2+} (Jahr and Stevens, 1987; Mayer et al., 1987; Ascher and Nowak, 1988), make them ideally suited to converting changes in synaptic activity into intracellular Ca^{2+} signals (Bliss and Collingridge, 1993).

Recently, interest has grown in the possibility that metabotropic L-glutamate (mGlu) receptors could also act as coincidence detectors by generating supralinear Ca^{2+} signals during membrane depolarization (Emptage, 1999; Nakamura et al., 1999). The physiological relevance of such interactions is indicated by findings that synaptic activity can lead to mGlu receptor-dependent Ca^{2+} signals (Frenguelli et al., 1993; Miller et al., 1996; Takechi et al., 1998; Nakamura et al., 1999; Yeckel et al., 1999) and that inhibition of mGlu receptors can, under some experimental conditions, result in blockade of the induction of LTP and LTD (Bortolotto et al., 1999). It is therefore important to understand the mechanisms that enable mGlu receptor-mediated supralinear Ca^{2+} signaling.

Received April 25, 2000; revised Sept. 6, 2000; accepted Sept. 15, 2000.

This work was supported by Wellcome Trust Grant 47368.

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In the present study, we have used Ca^{2+} imaging and whole-cell recording techniques to investigate the interactions between depolarization and/or NMDA receptor activation with mGlu receptors in the mediation of supralinear Ca^{2+} signals in cultured hippocampal neurons. We find that activation of $mGlu_5$ receptors in these cells releases substantial amounts of Ca^{2+} from intracellular stores, provided that they have been charged with Ca^{2+}. There is also an initial delay while the Ca^{2+} stores charge, and the enhancement persists for a period after removal of the store-loading stimulus. We find that the Ca^{2+} that is required to load the stores can enter either through L-type voltage-gated Ca^{2+} channels (VGCCs) or directly through NMDA receptors. This ménage à trois, in which depolarization can facilitate both NMDA and mGlu receptor Ca^{2+} signaling whereas NMDA receptor activation can facilitate mGlu receptor Ca^{2+} signaling, adds considerable flexibility to the processes involved in synaptic plasticity. In addition, the transient "memory" of previous neuronal activity contained within the Ca^{2+} stores could act as a mechanism for recency detection.

MATERIALS AND METHODS

Materials. The following materials were used: D(−)-2-amino-5-phosphonopentanoic acid (D-AP-5), (*RS*)-3,5-dihydroxyphenylglycine (DHPG), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX), and 2-methyl-6-(phenylethynyl)-pyridine (MPEP) were obtained from Tocris Cookson (Bristol, UK); cytosine arabinofuranoside, dialyzed fetal bovine serum, EGTA, fura-2, fura-2 AM, fluo-3 AM, thapsigargin, nifedipine, ionomycin, pronase E, protease type X, and tetrodotoxin were purchased from Sigma (Dorset, UK); minimal essential medium was purchased from Life Technologies (Paisley, UK).

Cell culture. Cultures of rat hippocampal neurons were prepared as described previously (Richmond et al., 1996). Briefly, rat pups (2 d old) were killed by cervical dislocation. After dissection, hippocampal tissue was treated with a mixture of pronase E and protease type X (both at 0.5 mg/ml) for 30 min in a HEPES-buffered saline (HBS) of the following composition (in mM): NaCl 130, HEPES 10, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, and D-glucose 25, pH 7.4. The tissue was dissociated by trituration, centrifuged, and then plated onto culture dishes (35 mm) that had been pretreated with poly-L-lysine (20 mg/ml for 3 hr). Cultures were then incubated at 37°C in medium consisting of 90% minimal essential medium, 10% dialyzed fetal bovine serum, and 2 mM L-glutamine. Cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C for up to

3 weeks. After 3–5 d in culture, cytosine arabinofuranoside (final concentration of 5 μM) was added to inhibit glial cell proliferation.

Dye loading and subsequent experiments were performed in HBS at room temperature. Experiments involving NMDA were performed in Mg^{2+}-free medium with 10 μM glycine. Culture dishes with attached cells were incubated with the Ca^{2+}-sensitive dyes fura-2 AM (6 μM; 40–60 min) or fluo-3 AM (10 μM; 40–60 min). To block indirect actions of mGlu receptor activation via synaptically driven Ca^{2+} transients, all experiments were performed in the presence of tetrodotoxin (0.5 μM). Compounds were applied directly to the perfusate (1.5–2 ml/min). Cells were studied between 6 and 21 d in culture. Data were obtained from neurons and glia, which were identified by their morphological and functional characteristics, including sensitivity to NMDA and depolarization with high extracellular K^+.

Microscopy and data analysis. In most experiments, a standard, conventional imaging system [PerkinElmer Life Sciences (Santa Clara, CA) or Improvision, Lexington, MA] and the Ca^{2+}-sensitive dye fura-2 were used to measure changes in intracellular Ca^{2+} levels. Ratiometric images (350/380 or 360/380 nm excitation) were collected at 3–10 sec intervals. This was increased to 0.5–1 sec intervals during mGlu receptor-mediated responses. Ratio values were calculated for each pixel in the frame, after subtraction of background fluorescence intensities. Numerical data were derived from somatic measurements unless otherwise indicated. In some experiments, the ratio values have been converted to estimated measurements of intracellular Ca^{2+} concentration using a method similar to that of Irving and Collingridge (1998) and the equations of Grynkiewicz et al. (1985). The calibration was performed *in situ*, in which R_{max}, R_{min}, and β values were determined using solutions containing a Ca^{2+} ionophore (10 μM ionomycin) with either nominally Ca^{2+}-free saline plus 1 mM EGTA or saline containing 1.8 mM Ca^{2+}. Because of the uncertainties associated with the accurate calibration of Ca^{2+} levels in cells, some of the data are presented as changes in fluorescence ratio rather than intracellular Ca^{2+} concentrations. High-resolution confocal imaging was performed using a Bio-Rad (Hercules, CA) Microradiance imaging system connected to an Olympus Optical (Tokyo, Japan) BX50 WI microscope (60× objective). Cells were loaded with the single wavelength, intensity modulating Ca^{2+} indicator fluo-3AM and were excited at 488 nm with emission detected at 550 nm. Data were analyzed both on-line and off-line using Time-Course software (Bio-Rad). Experiments were performed on at least two sets of cultures obtained from different rats, with up to 30 neurons being analyzed per experiment. Statistical analysis was performed using a paired Student's *t* test. $p < 0.05$ was considered significant. In protocols investigating the effects of blocking agents on responses to DHPG, only cells that exhibited <25% variability between two successive control responses were selected for analysis.

Electrophysiology. Whole-cell recordings were made at room temperature (22–25°C) from neurons using an Axopatch-200B amplifier (Axon Instruments, Foster City, CA) with patch pipettes of 3–10 MΩ resistance. Pipette solutions contained (in mM): KCl 20, K-gluconate 115, MgATP 4, GTP 0.3, phosphocreatine 10, HEPES 10, and fura-2 0.15, pH adjusted to 7.2 with KOH. The bathing solution contained (in mM): NaCl 130, HEPES 10, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, and D-glucose 25, pH 7.4. Experiments involving NMDA were performed in Mg^{2+}-free medium with 10 μM glycine. Cells were initially held at −60 mV and were low-pass filtered at 1–5 kHz. Series resistances ranged from 15 to 30 MΩ and were not compensated. Solution changes were achieved using a gravity feed system at a rate of 1.5–2 ml/min.

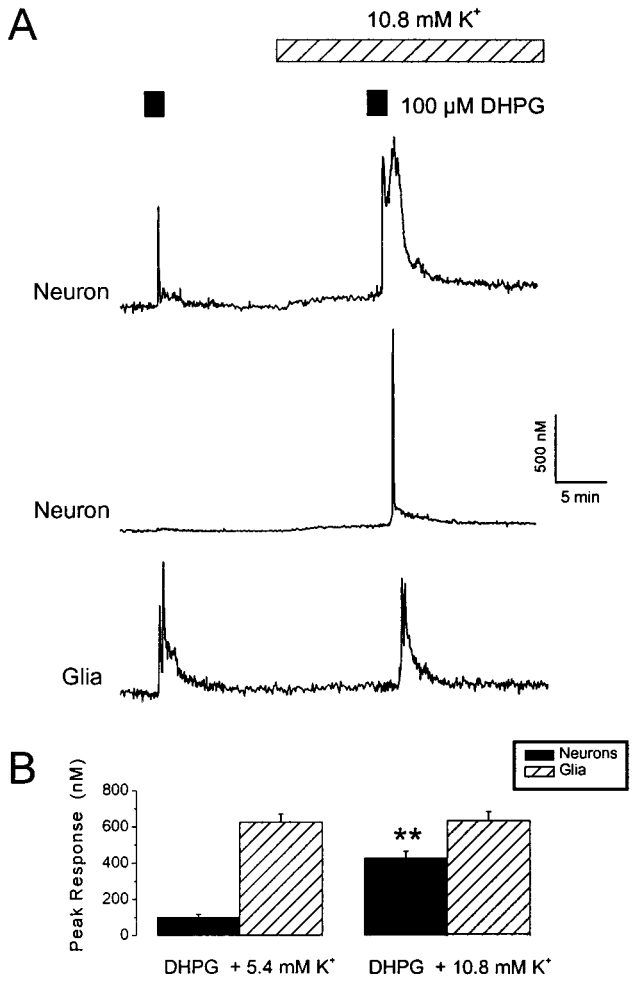

Figure 1. Neuronal responses to DHPG are enhanced in the presence of elevated extracellular K^+. *A*, Traces showing changes in intracellular Ca^{2+} levels within the somas of two neurons and a glial cell. Cells were exposed to DHPG under control conditions (100 μM; 1.5 min exposure; 5.4 mM extracellular K^+) or in the presence of a moderate elevation in the extracellular K^+ concentration (total of 10.8 mM). Note the marked enhancement of the neuronal response to DHPG with minimal effects of elevated K^+ on resting Ca^{2+} levels. *B*, A histogram illustrating pooled data for the effects of elevated extracellular K^+ levels on DHPG responses in neurons (*black bars*) and glia (*hatched bars*). $^{**}p < 0.01$.

RESULTS

Activation of mGlu receptors combined with depolarization generates supralinear Ca^{2+} signals

The group I mGlu receptor agonist DHPG (100 μM; applied for 90 sec) (Conn and Pin, 1997) elicited little (<100 nM; 31 of 68 cells) or no (22 of 68 cells) increase in intracellular Ca^{2+} levels under control conditions (5.4 mM K^+; 1.8 mM Ca^{2+}) in the majority of hippocampal neurons tested. When present, neuronal responses to DHPG comprised a single Ca^{2+} transient, with a rapid rising phase and slower decay. Reproducible responses to DHPG could be obtained providing sufficient time separated successive agonist applications (10–15 min).

To determine whether DHPG-induced responses were facilitated by membrane depolarization, we doubled the extracellular K^+ concentration to 10.8 mM. This proved remarkably effective, such that DHPG induced responses in almost all neurons investigated (60 of 68 cells). Indeed, in a proportion of these neurons, evoked responses were only observed in the presence of elevated extracellular K^+ (19 of 68 cells). Overall, DHPG-induced responses in the presence of 10.8 mM K^+ were increased from a mean ± SEM peak amplitude of 87 ± 21 to 322 ± 45 nM ($p < 0.01$) (Fig. 1). Doubling the extracellular K^+ concentration usually resulted in a small increase in intracellular Ca^{2+} levels (90 ± 8 nM above basal; $p < 0.01$; $n = 60$). In neurons in which there was no detectable elevation in intracellular Ca^{2+} levels during exposure to 10.8 mM K^+, a marked enhancement of responses to DHPG was still observed (Fig. 1*A*). Thus, the combination of DHPG with a modest K^+ elevation generated a marked, supralinear increase in intracellular Ca^{2+} levels, in which the peak Ca^{2+} rise was much greater than the summation of the individual responses.

The temporal profile of mGlu receptor-mediated responses was also modified in the presence of elevated K^+. The duration of the response to DHPG in neurons was usually prolonged, and, in some cells, pronounced oscillatory responses were observed. In addition, the latency between agonist exposure and response was reduced; the time from the start of perfusion with DHPG to 50% of the peak response was 40 ± 3 sec in 5.4 mM K^+ and 27 ± 3 sec in 10.8 mM K^+ ($n = 15$; these values include a dead space time of ~20 sec; $p < 0.01$). A separate series of experiments, using confocal imaging of fluo-3 AM-loaded cells, investigated the cellular localization of mGlu receptor-mediated responses under optimal conditions for the release of Ca^{2+} from intracellular stores. In the presence of 10.8 mM K^+, DHPG-induced responses were observed throughout the neuron, including fine processes ($n = 6$; data not shown).

In contrast to the data obtained with the neurons under control conditions, DHPG elicited much larger responses in glial cells

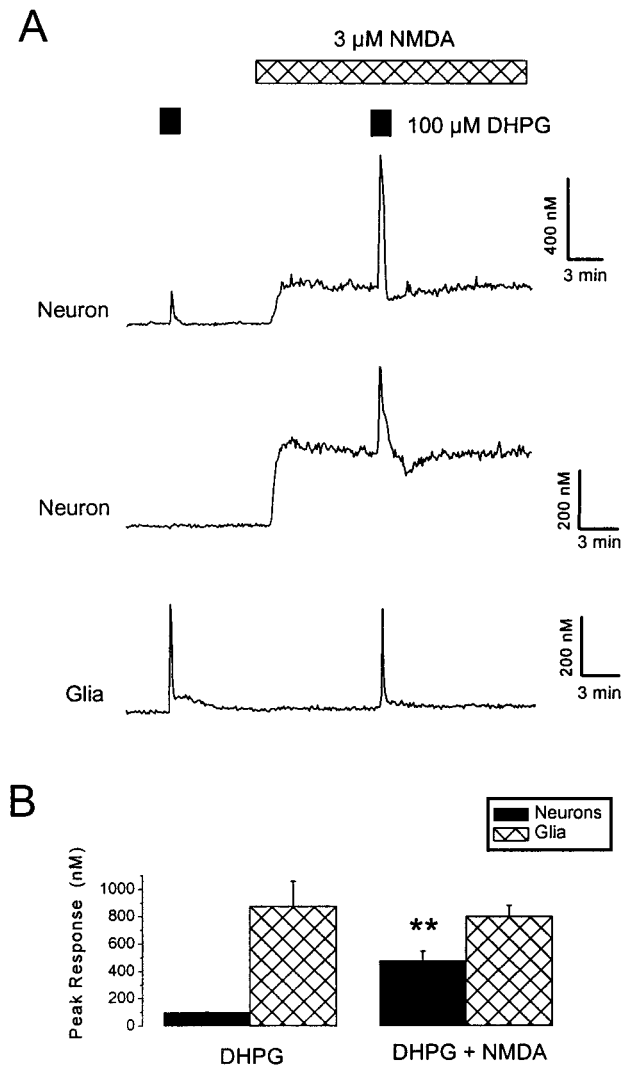

Figure 2. Supralinear interactions between NMDA and DHPG receptor-mediated Ca^{2+} signals. *A*, DHPG (100 μM) was applied under control conditions and in the presence of NMDA (3 μM). Neuronal, but not glial, responses to DHPG were greatly enhanced during exposure to NMDA. The *middle trace* illustrates a neuron that only exhibited a response to DHPG in the presence of NMDA. *B*, A histogram illustrating pooled data for the effects of NMDA on DHPG responses in neurons (*black bars*) and glia (*hatched bars*). $^{**}p < 0.01$.

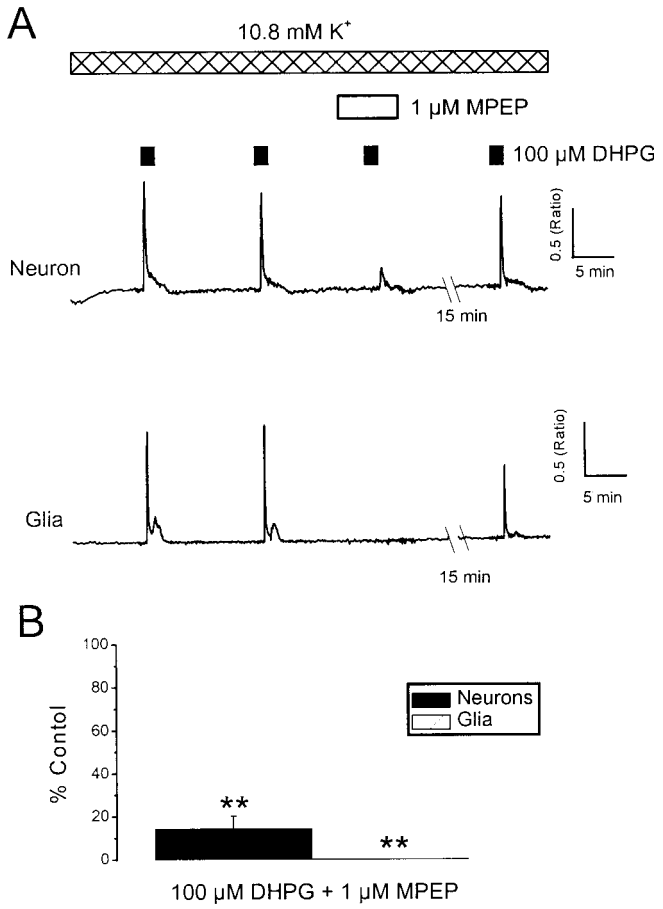

Figure 3. Antagonism of DHPG responses by the mGlu receptor antagonist MPEP. *A*, Traces from a neuron and a glial cell that were exposed to successive applications of DHPG (100 μM) in the presence of 10.8 mM K^+. Responses to DHPG were blocked in the presence of MPEP (1 μM) and recovered on washout. *B*, A histogram illustrating pooled data for the effects on DHPG responses in neurons (*black bars*) and glia (*hatched bars*). $^{**}p < 0.01$.

present in the same cultures (624 ± 47 nM; $n = 21$) (Fig. 1). Glial responses consisted of an initial spike, which was usually followed by a plateau phase and an oscillatory Ca^{2+} signal. Intracellular Ca^{2+} levels in glial cells were not significantly affected by doubling extracellular K^+ levels ($p > 0.05$; $n = 31$), and glial responses to DHPG were not facilitated by this treatment ($p > 0.05$; $n = 31$) (Fig. 1). Thus, in this preparation, the combination of mGlu receptor activation and elevated extracellular K^+ levels generated supralinear Ca^{2+} signals in neurons but not glia.

Supralinear interactions between mGlu and NMDA receptors

We also studied interactions between NMDA receptor-mediated Ca^{2+} influx and DHPG-induced Ca^{2+} release. Exposure of cells to NMDA (3 μM) elevated intracellular Ca^{2+} levels in neurons by 228 ± 18 nM ($p < 0.01$; $n = 47$) but was without effect in glia ($p > 0.5$; $n = 13$). In neurons, the combined presence of NMDA and DHPG greatly enhanced the intracellular Ca^{2+} response (Fig. 2). Moreover, in seven neurons that exhibited no detectable response to a DHPG exposure under control conditions, a response to DHPG was observed in the presence of NMDA (3 μM). Overall, the mean, peak neuronal response to DHPG in control medium was 107 ± 8 nM, whereas in the presence of NMDA, it was 478 ± 70 nM ($p < 0.05$; 45 cells). As with exposure to high extracellular K^+, the temporal profile of DHPG responses was modified in the presence of NMDA. The duration of responses to DHPG in neurons was prolonged, and, in some cells, pronounced oscillatory responses were observed. In glial cells, NMDA had no significant effect on the magnitude of responses to DHPG ($p > 0.05$; $n = 13$). Thus, the effects of elevated extracellular K^+ and NMDA on DHPG-induced responses were very similar.

DHPG responses are attributable to $mGlu_5$ receptor-mediated Ca^{2+} mobilization

Group I mGlu receptors comprise two subtypes, $mGlu_1$ and $mGlu_5$, both of which are expressed in hippocampal neurons (Conn and Pin, 1997). We found that DHPG responses, evoked in the presence of elevated K^+, were greatly inhibited in neurons (by 86 ± 6%; $p < 0.01$; $n = 18$) and abolished in glia ($p < 0.01$; $n = 16$) by the specific $mGlu_5$ receptor antagonist MPEP (1 μM) (Gasparini et al., 1999) (Fig. 3). A combination of the ionotropic glutamate receptor antagonists NBQX (2 μM) and D-AP-5 (50 μM) had no significant effect on the magnitude of responses to DHPG in the presence of 10.8 mM K^+ ($n = 11$; $p > 0.05$). Thus, $mGlu_5$ receptors are the predominant subtype involved in the present investigation.

DHPG responses evoked in the presence of either elevated extracellular K^+ or NMDA are reminiscent of Ca^{2+} release from intracellular stores (Murphy and Miller, 1989; Irving and Col-

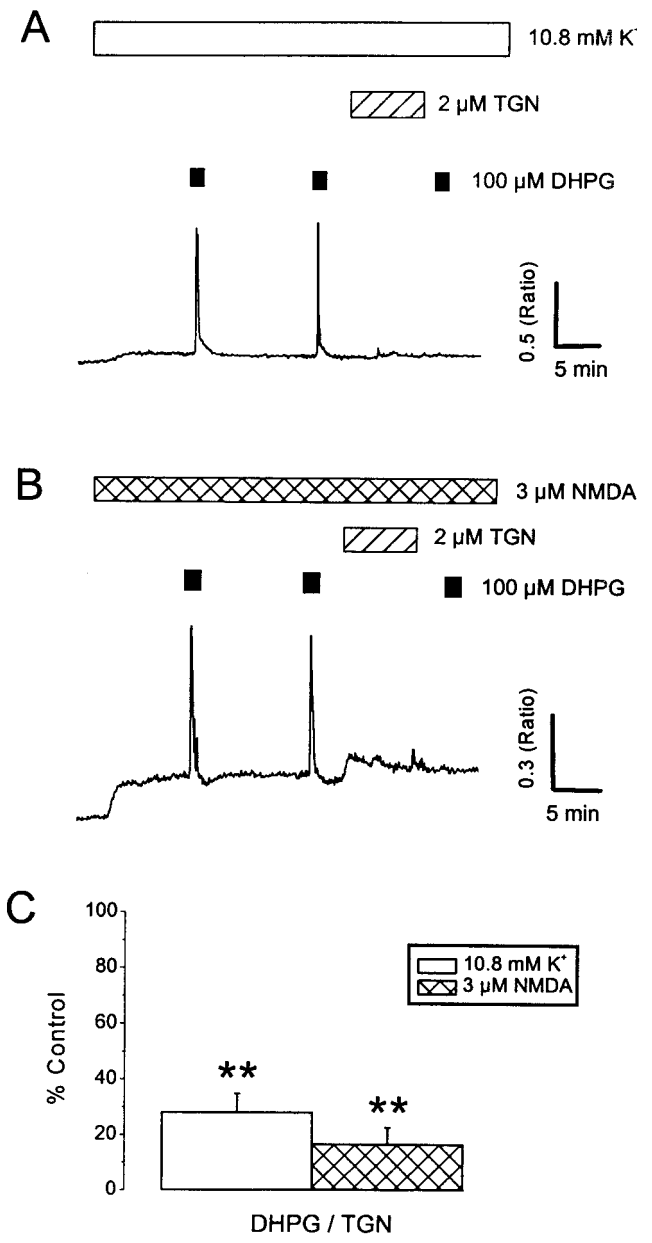

Figure 4. Involvement of Ca^{2+} stores in the supralinear responses. *A*, Neurons were exposed to successive applications of 100 μM DHPG in the presence of 10.8 mM K^+ (*A*) or NMDA (3 μM; *B*). Treatment with thapsigargin (*TGN*; 2 μM; 8 min) blocked subsequent responses to DHPG. *C*, A histogram illustrating pooled data for the effects of thapsigargin on DHPG responses in the presence of 10.8 mM K^+ (*white bars*) and NMDA (*hatched bars*). $^{**}p < 0.01$.

lingridge, 1998). This was confirmed using thapsigargin (1 μM) (Fig. 4), an agent that irreversibly depletes intracellular Ca^{2+} stores by inhibition of the sarcoplasmic/endoplasmic Ca^{2+} ATPase (SERCA) (Law et al., 1990) and prevents Ca^{2+} mobilization after their spontaneous emptying (Irving et al., 1992). Responses to DHPG in the presence of elevated K^+ were inhibited by 72 ± 7% ($p < 0.01$; $n = 23$) in neurons and by 97 ± 3% ($p < 0.01$; $n = 9$) in glia after exposure to thapsigargin (2 μM; applied for 8 min). Likewise, neuronal responses to DHPG in the presence of NMDA were inhibited by 83 ± 6% ($p < 0.01$; $n = 20$) after exposure to thapsigargin.

Treatment of neurons with thapsigargin during exposure to 10.8 mM K^+ or NMDA was only occasionally linked with a small elevation in intracellular Ca^{2+} levels, which slowly returned to baseline levels. This suggests three things: first, that Ca^{2+} release from intracellular stores is not contributing to the modest increases in Ca^{2+} levels that are associated with exposure to high extracellular K^+ or NMDA; second, that the stores discharge spontaneously; and third, that this occurs sufficiently slowly for the Ca^{2+} to be rapidly excluded from the cytosol (Garaschuk et al., 1997). In glial cells, thapsigargin induced a marked elevation in intracellular Ca^{2+} levels that decayed slowly but remained at a level above the initial baseline (data not shown). This sustained increase in Ca^{2+} levels presumably reflects the presence of a powerful store-operated Ca^{2+} influx pathway in glia (Simpson and Russell, 1997). However, the absence of a similar Ca^{2+} elevation in neurons does not rule out the presence of a store-operated Ca^{2+} influx pathway in these cells.

Neuronal Ca^{2+} stores have a high capacity to sequester Ca^{2+}

The previous experiments suggest that Ca^{2+} stores are the primary source of the supralinear response when DHPG was combined with depolarization or NMDA exposure. Under these conditions, the Ca^{2+} content of neuronal Ca^{2+} stores was tested using a high concentration of caffeine (50 mM) to directly activate Ca^{2+}-induced, Ca^{2+} release channels through a mechanism that is independent of cytosolic Ca^{2+} levels (Sitsapesan and Williams, 1990; Irving and Collingridge, 1998). A brief exposure of neurons to caffeine (90 sec) under control conditions elicited little (<100 nM; 30 of 59 cells) or no (17 of 59 cells) increase in intracellular Ca^{2+} levels. However, in the presence of 10.8 mM K^+ or NMDA (3 μM; Mg^{2+}-free medium), responses to caffeine were markedly enhanced (Fig. 5*A–C*) and were observed in almost all neurons investigated ($n = 53$ of 59). Overall, caffeine responses were increased from a mean peak amplitude of 52 ± 10 to 199 ± 31 nM ($p < 0.01$; $n = 31$) in the presence of 10.8 mM K^+ and from 69 ± 12 to 281 ± 38 nM in the presence of NMDA (3 μM; $p < 0.01$; $n = 28$). In control experiments, ryanodine (10 μM) abolished responses to caffeine (50 mM; $n = 6$). Caffeine elicited no detectable Ca^{2+} rise in glial cells under any condition ($n = 40$).

These data indicate that Ca^{2+} stores are functionally depleted under control conditions. If the supralinear responses reflect the loading of Ca^{2+} stores, then the potentiation would also be expected to persist for a period after a store-loading paradigm and there would be an initial delay in the enhancement of responses to DHPG as the stores charge with Ca^{2+}. Thus, the magnitude of responses to DHPG was compared after various times in the presence of, or after, depolarization with 10.8 mM K^+. A delay of 2–8 min was observed before maximal levels of potentiation were achieved after exposure to elevated K^+, and a degree of facilitation persisted for up to 10 min after removal of the store-loading stimulus (Fig. 5*D–F*). These results are consistent with supralinear Ca^{2+} signaling reflecting the loading state of Ca^{2+} stores.

Role of L-type voltage-gated Ca^{2+} channels

Because in neurons the state of filling of Ca^{2+} stores has such an influence on the DHPG-induced response, it is important to determine the pathways by which the Ca^{2+} stores can be filled. The influx of Ca^{2+} through L-type VGCCs has been reported previously to charge Ca^{2+} stores during depolarization (Irving and Collingridge, 1998). Therefore, the contribution of L-type VGCCs to the loading of DHPG-sensitive intracellular Ca^{2+} stores under control conditions, in the presence of elevated extracellular K^+ or NMDA (3 μM), was investigated. The L-type Ca^{2+} channel antagonist nifedipine (10 μM) did not significantly affect either neuronal ($n = 12$) or glial ($n = 6$) responses to DHPG obtained under control conditions ($p > 0.05$). However, responses in the presence of elevated K^+ were strongly inhibited in its presence (83 ± 9% inhibition; $n = 9$; $p < 0.01$) (Fig. 6). In contrast, responses to DHPG in the presence of NMDA were only slightly reduced by nifedipine (13 ± 9% inhibition; $n = 9$; $p < 0.05$) (Fig. 6). Nifedipine had little effect on resting Ca^{2+} levels in control medium but lowered intracellular Ca^{2+} levels to near control values in which an increase in Ca^{2+} was observed during exposure to 10.8 mM K^+. In

the presence of NMDA, nifedipine only reduced Ca^{2+} levels slightly (Fig. 6). These results suggest that the same Ca^{2+} stores can be filled by at least three separate pathways; one that is active at rest, and one involving L-type Ca^{2+}, which accounts for most of the effect of a small depolarization and a pathway that is mainly used after activation of NMDA receptors.

Two activity-dependent mechanisms for Ca^{2+} store loading

To confirm that postsynaptic depolarization alone is sufficient to load Ca^{2+} stores, experiments were performed using combined whole-cell recording and Ca^{2+} imaging. In current-clamp experiments, exposure of neurons to 10.8 mM K^+ resulted in a small depolarization of 9.6 ± 0.6 mV from a mean resting potential of −52 ± 1.7 mV ($n = 12$). Thus, the ability of modest depolarization to enhance DHPG responses was tested directly under voltage-clamp conditions. In seven neurons, responses to DHPG were enhanced from 0.33 ± 0.12 to 0.64 ± 0.14 ratio units ($p < 0.05$; $n = 10$) by a 15–30 mV depolarization, relative to the initial holding potential of −60 mV (Fig. 7*A*).

The nifedipine-insensitive enhancement of DHPG responses in the presence of NMDA could be attributable to activation of a different class of voltage-gated Ca^{2+} channel(s) or the result of Ca^{2+} permeation through NMDA channels. This was tested directly by holding cells at −60 mV throughout the experiment. Responses to DHPG were enhanced from 0.36 ± 0.17 to 0.69 ± 0.14 ratio units ($p < 0.05$; $n = 10$) in the presence of NMDA (3 μM) (Fig. 7*B*).

Collectively, these data show that activation of $mGlu_5$ receptors can release Ca^{2+} from intracellular stores. These stores are functionally depleted at rest but can be charged with Ca^{2+} either via modest depolarization, which activates L-type Ca^{2+} channels, or by Ca^{2+} entry through NMDA receptors (Fig. 8).

DISCUSSION

Supralinear Ca^{2+} responses are observed in neurons when mGlu receptor activation is combined with depolarization or exposure to NMDA, and this interaction reflects the loading state of intracellular Ca^{2+} stores. Group I mGlu receptor-mediated responses are also observed in glial cells (Bernstein et al., 1998); however, they are not modulated by depolarization or exposure to NMDA.

Multiple pathways for loading Ca^{2+} stores

The reduced magnitude of neuronal Ca^{2+} mobilizing responses under control conditions suggests that the Ca^{2+} stores are only partially loaded (Garaschuk et al., 1997) or functionally empty (Shmigol et al., 1996; Irving and Collingridge, 1998; Koizumi et al., 1999) at rest. Reproducible responses to DHPG were observed in glia under control conditions, suggesting that their Ca^{2+} stores are able to replenish spontaneously (Simpson and Russell, 1997). When present, reproducible responses were also observed in neurons at rest. In many types of nonexcitable cells, store depletion triggers a Ca^{2+} release-activated Ca^{2+} current (I_{CRAC}) (Parekh

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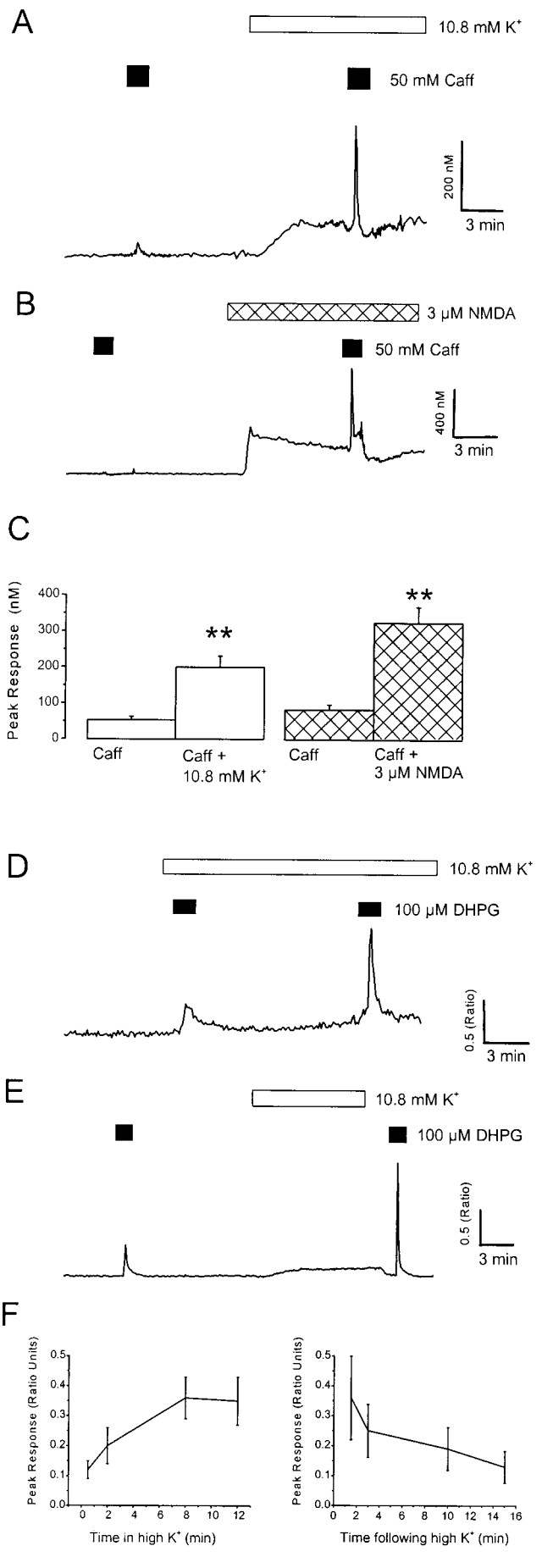

Figure 5. Loading and unloading neuronal Ca^{2+} stores. *A–C*, The Ca^{2+} content of neuronal Ca^{2+} stores was tested using a high concentration of caffeine. *A*, The neuron was exposed to caffeine (*Caff*; 50 mM) in control medium and then in the presence of high extracellular K^+ (10.8 mM). *B*, The neuron was exposed to caffeine in Mg^{2+}-free medium and then in the presence of NMDA (3 μM). Note the similar enhancement of neuronal responses to caffeine during exposure to elevated extracellular K^+ or NMDA. *C*, A histogram illustrating pooled data for the effects of 10.8 mM K^+ (*white bars*) and NMDA (*hatched bars*) on caffeine responses. $^{**}p < 0.01$. These data indicate that neuronal Ca^{2+} stores have a high capacity to sequester Ca^{2+}. *D–F*, Time course of loading and discharging of Ca^{2+} stores. *D*, The cell was exposed to 100 μM DHPG close to the onset (30 sec) of perfusion with elevated K^+-containing medium (10.8 mM) and then applied 12 min later. *E*, The cell was exposed to DHPG under control conditions and then a short period (90 sec) after removal of elevated K^+ (applied for 10 min). *F*, Pooled data illustrating the effects of duration of exposure to 10.8 mM K^+ ($n = 19$) and time after removal of 10.8 mM K^+ on the magnitude of responses to DHPG ($n = 11$).

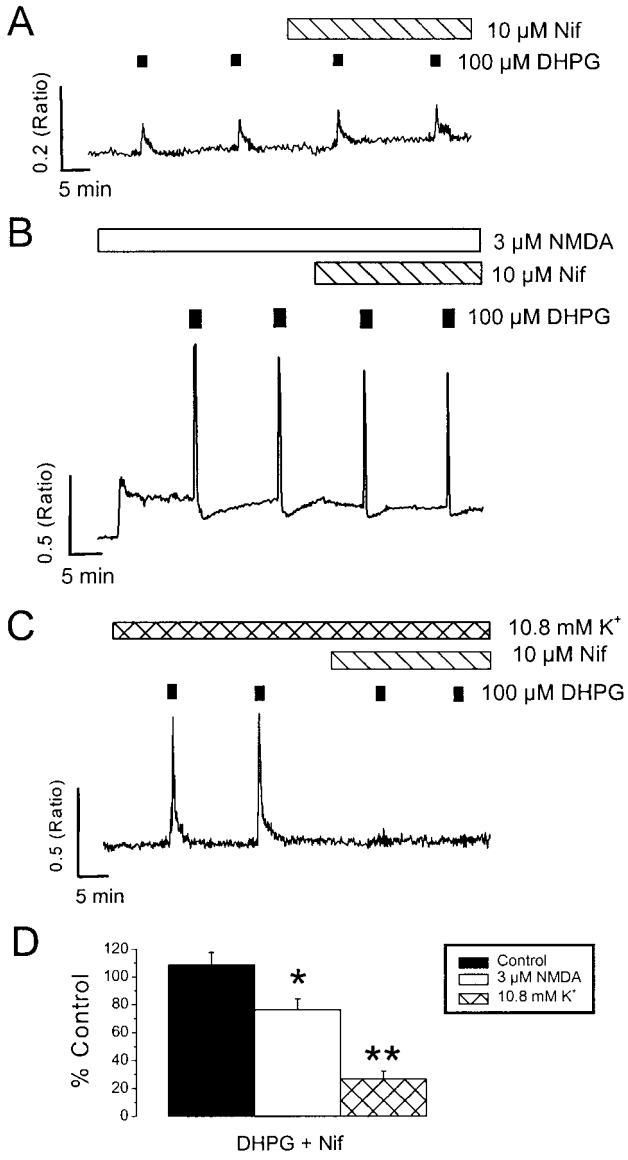

Figure 6. Role of L-type voltage-gated Ca^{2+} channels in charging Ca^{2+} stores. Neurons were exposed to four successive applications of DHPG (100 μM) in control media (*A*), in the presence of NMDA (3 μM; *B*), or in the presence of 10.8 mM K^+ (*C*). The final two DHPG applications were made in the presence of nifedipine (*Nif*; 10 μM). *D*, A histogram illustrating pooled data for the effects of nifedipine on responses to DHPG in control media (*black bars*), in the presence of NMDA (3 μM; *white bars*), or in the presence of 10.8 mM K^+ (*hatched bars*). $^*p < 0.05$; $^{**}p < 0.01$. Note the strong inhibition of responses to DHPG obtained in the presence of 10.8 mM K^+ by nifedipine. Only neurons that responded to DHPG were selected for analysis.

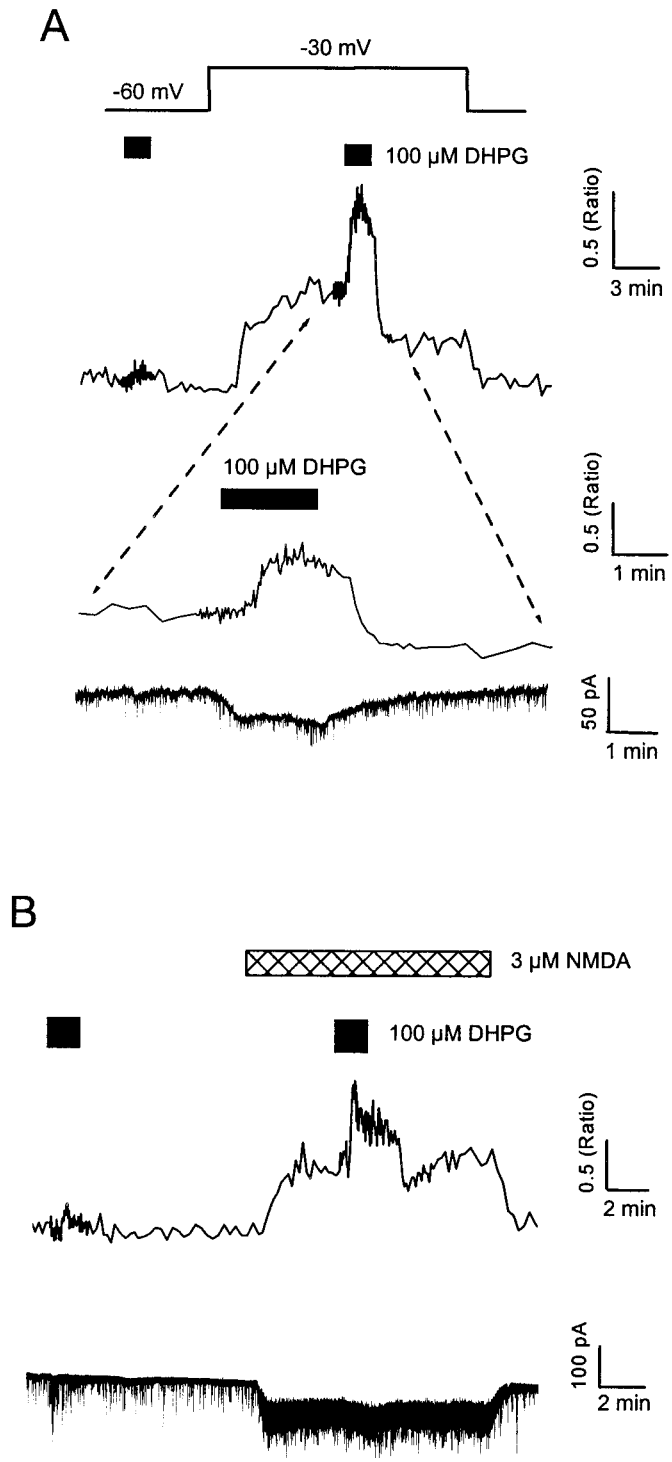

Figure 7. Multiple pathways for loading neuronal Ca^{2+} stores. *A*, *B*, Combined whole-cell voltage-clamp recordings and Ca^{2+} imaging, with fura-2 (150 μM) in the patch pipette. *A*, The neuron was exposed to 100 μM DHPG at a holding potential of −60 mV, the holding potential was then changed to −30 mV, as indicated by the voltage step, and DHPG was reapplied. The expanded region shows the Ca^{2+} response to DHPG during depolarization (*top trace*) associated with a small inward current (*bottom trace*). Similar currents were present in other cells and ranged from 3 to 45 pA. Note the absence of a detectable Ca^{2+} response to DHPG at −60 mV. *B*, The neuron was exposed to 100 μM DHPG at a holding potential of −60 mV in control Mg^{2+}-free media and then in the presence of NMDA (3 μM). Exposure to NMDA itself was associated with an inward current (40.1 ± 10.8 pA; $n = 10$) and an increase in membrane noise. The spontaneous, spike-like activity on the electrical traces represent miniature synaptic currents.

and Penner, 1997), which is thought to underlie "capacative" Ca^{2+} influx and store refilling (Putney, 1986). In addition, a novel non-capacitative Ca^{2+} entry channel (I_{ARC}) has been described recently in human embryonic kidney cells (Mignen and Shuttleworth, 2000), which could also allow store refilling during pulsatile Ca^{2+} release. Similar pathways may be involved in store refilling after Ca^{2+} release under resting conditions in mammalian neurons (Garaschuk et al., 1997; Usachev and Thayer, 1999).

Neurons express many different types of Ca^{2+}-permeable channels in their plasma membranes, which could, in principle, link with their Ca^{2+} stores. The present data show that activation of VGCCs or NMDA receptors can add substantial additional loading to neuronal Ca^{2+} stores. The involvement of L-type Ca^{2+} channels in the depolarization-induced loading of IP_3-sensitive Ca^{2+} stores has been implicated in our previous work characterizing musca-

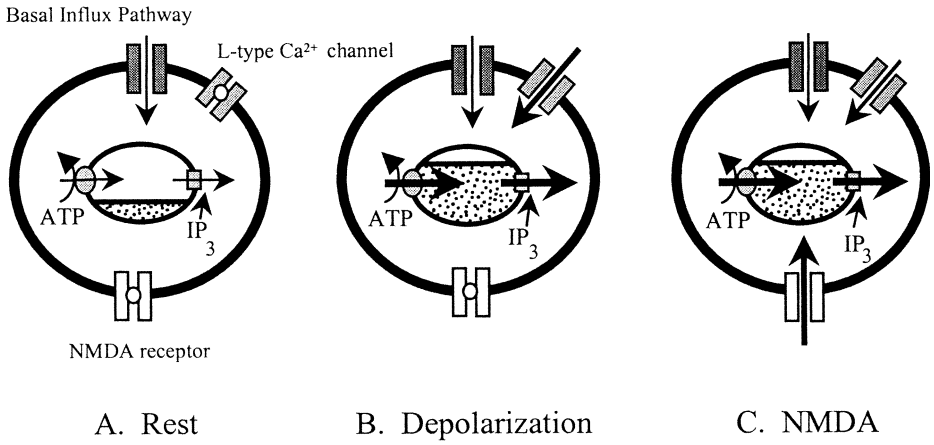

Figure 8. Scheme illustrating the different pathways for loading IP_3-sensitive Ca^{2+} stores in hippocampal neurons. *A*, At rest, background loading of Ca^{2+} stores is via a dihydropyridine-insensitive basal influx pathway. Activity-dependent Ca^{2+} influx via L-type VGCCs during depolarization (*B*) or by stimulation of NMDA receptor-operated channels (*C*) substantially increases the Ca^{2+} loading of the stores and allows a strong Ca^{2+} signal on activation of IP_3 receptors. The ability of IP_3-linked agonists to mobilize Ca^{2+} is primarily related to the level of Ca^{2+} contained within the stores, which is governed by the net flux of Ca^{2+} into the cell, the activity of the SERCA pump, and by the activity of the release channels.

rinic responses in cultured hippocampal neurons (Irving and Collingridge, 1998). The lack of dihydropyridine sensitivity of neuronal DHPG responses under control conditions is similar to that observed with responses to caffeine in hippocampal slices (Garaschuk et al., 1997). The enhancement of caffeine responses by depolarization or NMDA receptor activation extends on previous studies investigating the properties of caffeine-sensitive Ca^{2+} stores (Friel and Tsien 1992; Shmigol et al., 1994; Garaschuk et al., 1997; Koizumi et al., 1999). The present results can be explained by the development of a simple scheme (Irving and Collingridge, 1998), whereby the loading state of the intracellular Ca^{2+} stores in neurons is regulated dynamically by Ca^{2+} influx across the plasma membrane (Fig. 8) (see also Verkhratsky and Petersen, 1998).

Supralinear Ca^{2+} signaling

Classically, the biophysical properties of NMDA receptors enable them to act as coincidence detectors (Mayer et al., 1984; Nowak et al., 1984). The present data suggests that the group I mGlu receptor-mediated Ca^{2+} signaling could also detect changes in neuronal activity by sensing membrane depolarization or NMDA receptor activation. Although this functionality can arise at a number of different levels, the loading state of intracellular Ca^{2+} stores represents the primary mechanism underlying this action. There are two inter-related processes whereby Ca^{2+} stores could influence the response to phosphoinositidase C (PIC)-linked agonists. The magnitude of the response will be dependent on the quantity of Ca^{2+} available for release, and the sensitivity of Ca^{2+} release channels may themselves be regulated by luminal Ca^{2+} levels (Missiaen et al., 1992; Koizumi et al., 1999). Indeed, increased store loading in PC12 cells leads to an enhancement in the frequency and coupling between elementary release sites leading to the generation of global Ca^{2+} signals (Koizumi et al., 1999). An action of luminal Ca^{2+} on the sensitivity of neuronal Ca^{2+} release channels is also indicated by the finding that relatively short periods of thapsigargin exposure, under conditions in which Ca^{2+} stores are fully charged, can block subsequent responses to DHPG without necessarily increasing Ca^{2+} levels. Because the period of exposure to thapsigargin is relatively short, the stores themselves are likely to be only partially depleted. However, this effect is presumably sufficient to inhibit IP_3-mediated Ca^{2+} release.

The contribution of other processes, such as the effects of cytosolic Ca^{2+} on the sensitivity of the IP_3 receptor (Bezprozvanny et al., 1991, Nakamura et al., 1999) and PIC activation (Irving et al., 1992; Challiss et al., 1994), could also have a role. The observation that there is a reduction in the response latency to DHPG in elevated K^+ relative to that in control medium may indicate higher levels of IP_3 formation (Carter and Ogden, 1997). Importantly, however, the marked facilitation of responses to DHPG by modest depolarization does not necessarily require increased cytosolic Ca^{2+} levels. This suggests further that the loading of intracellular stores is the key factor in the observed supralinearity. For example, the enhancement of DHPG responses for a period after depolarization is indicative of store loading (Garaschuk et al., 1997; Irving and Collingridge, 1998) and is present when Ca^{2+} levels have returned to baseline. In addition, the store-loading stimulus itself need not increase cytosolic Ca^{2+} levels. If during depolarization the buffering capacity of the cell (including Ca^{2+} pumps on the endoplasmic reticulum and plasma membrane) can effectively handle the increased Ca^{2+} influx, luminal Ca^{2+} levels will rise without an associated elevation in the cytoplasmic Ca^{2+} concentration (Irving and Collingridge, 1998). Moreover, the idea that loading (or refilling) of Ca^{2+} stores can occur without any measurable rise in cytosolic Ca^{2+} levels is supported by studies on pancreatic acinar cells (Mogami et al., 1997).

In pyramidal cells (Bianchi et al., 1999) and interneurons (Woodhall et al., 1999) in hippocampal slices, group I mGlu receptor activation can elevate Ca^{2+} levels by membrane depolarization and activation of VGCCs. It is possible that a component of the DHPG-induced Ca^{2+} rise observed in the present investigation could also be mediated by recruitment of VGCCs; however, a number of observations indicate that Ca^{2+} release from intracellular stores is the principle source of the Ca^{2+} elevation. Thus, DHPG responses were sensitive to thapsigargin, were observed under voltage-clamp conditions, and were only associated with small inward currents, which often exhibited a different time course from the Ca^{2+} transients. Brief exposure of cells to DHPG was not associated with the prolonged Ca^{2+} rises observed in pyramidal cells by Bianchi et al. (1999).

Activation of group I mGlu receptors can also directly enhance responses to NMDA through a mechanism that is independent of Ca^{2+} release from stores in rat hippocampal slices (Harvey and Collingridge, 1993; Fitzjohn et al., 1996) and augment mGlu receptor-mediated currents in CA3 pyramidal neurons in cultured slices (Lüthi et al., 1994). It is unlikely that this effect contributes much to the supralinear responses observed when DHPG was applied in the presence of NMDA because the responses were markedly inhibited after thapsigargin exposure. In addition, a similar interaction was observed when NMDA was combined with a high concentration of caffeine, which directly stimulates Ca^{2+} release from intracellular stores (Sitsapesan and Williams, 1990; Irving and Collingridge, 1998).

Physiological implications

In neurons, group I mGlu receptors may have a key role in detecting changes in neuronal activity through the generation of supralinear signals. This action reflects the characteristics of mGlu receptor-mediated Ca^{2+} signaling and the loading state of intracellular stores. Consequently, it need not be specific to mGlu receptors and could be exhibited by other PIC-coupled receptors in these cells (Irving and Collingridge, 1998). The global nature of the supralinear responses is ideally suited to conveying Ca^{2+} signals from neurites and spines to the nucleus (Berridge, 1998; Nakamura et al., 1999), which may be involved in changes in gene expression associated with synaptic plasticity (Stanton and Sarvey,

1984; Frey and Morris, 1997). Although depolarization itself can enhance Ca^{2+} influx through NMDA receptor-operated channels, the present data show that additional, powerful mechanisms exist for the generation of supralinear Ca^{2+} rises. A combination of NMDA and mGlu receptor activation leading to supralinear Ca^{2+} signaling may be involved during the induction of LTP in area CA1 of the hippocampus during weak stimulation paradigms, whereas NMDA receptor activation alone may be sufficient for synaptic plasticity during robust stimulation (Wilsch et al., 1998). A linkage between depolarization and mGlu receptor-mediated Ca^{2+} signaling could also act as a distinct mechanism for detecting changes in synaptic activity. Moreover, a synergistic interaction between depolarization and mGlu receptor-mediated Ca^{2+} release from stores may have a role in coincidence detection associated with backpropagating action potentials (Nakamura et al., 1999). The present data show that the mechanism of detection involving mGlu receptor-mediated Ca^{2+} signaling has different temporal properties compared with the NMDA receptor. There is an initial delay while the Ca^{2+} stores charge, and the effect persists for a period after removal of the store-loading stimulus. Thus, this process could act as a mechanism for recency detection by integrating activity that is close in time but not necessarily temporally coincident. In summary, the expression of multiple routes for the generation of supralinear Ca^{2+} signals adds considerable flexibility to the processes that underlie activity-dependent changes in synaptic strength.

REFERENCES

Alford S, Frenguelli BG, Schofield JG, Collingridge GL (1993) Characterization of Ca^{2+} signals induced in hippocampal CA1 neurones by the synaptic activation of NMDA receptors. J Physiol (Lond) 469:693–716.

Ascher P, Nowak L (1988) The role of divalent cations in the *N*-methyl-D-aspartate responses of mouse central neurones in culture. J Physiol (Lond) 399:247–266.

Bear MF, Abraham WC (1996) Long-term depression in the hippocampus. Annu Rev Neurosci 19:437–462.

Bernstein M, Behnisch T, Balschun D, Reymann KG, Reiser G (1998) Pharmacological characterisation of metabotropic glutamatergic and purinergic receptors linked to Ca^{2+} signalling in hippocampal astrocytes. Neuropharmacology 37:169–178.

Berridge MJ (1998) Neuronal calcium signaling. Neuron 21:13–26.

Bezprozvanny I, Watras J, Ehrlich BE (1991) Bell-shaped calcium-response curves of $Ins(1,4,5)P_3$- and calcium-gated channels from endoplasmic reticulum of cerebellum. Nature 351:751–754.

Bianchi R, Young SR, Wong RKS (1999) Group I mGluR activation causes voltage-dependent and-independent Ca^{2+} rises in hippocampal pyramidal cells. J Neurophysiol 81:2903–2913.

Bliss T, Collingridge G (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31–39.

Bortolotto ZA, Fitzjohn SM, Collingridge GL (1999) Roles of metabotropic glutamate receptors in LTP and LTD in the hippocampus. Curr Opin Neurobiol 9:299–304.

Carter TD, Ogden D (1997) Kinetics of Ca^{2+} release by $InsP_3$ in pig single aortic endothelial cells: evidence for an inhibitory role of cytosolic Ca^{2+} in regulating hormonally evoked Ca^{2+} spikes. J Physiol (Lond) 504:17–33.

Challiss RAJ, Mistry R, Gray DW, Nahorski SR (1994) Modulatory effects of NMDA on phosphoinositide responses evoked by the metabotropic glutamate receptor agonist 1*S*,3*R*-ACPD in neonatal rat cerebral cortex. Br J Pharmacol 112:231–239.

Conn PJ, Pin JP (1997) Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 37:205–237.

Emptage N, Bliss TV, Fine A (1999) Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. Neuron 22:115–124.

Emptage NJ (1999) Calcium on the up: supralinear calcium signaling in central neurons. Neuron 24:495–497.

Fitzjohn SM, Irving AJ, Palmer MJ, Harvey J, Lodge D, Collingridge GL (1996) Activation of group I mGluRs potentiate NMDA responses in rat hippocampal slices. Neurosci Lett 203:1–3.

Frenguelli BG, Potier B, Slater NT, Alford S, Collingridge GL (1993) Metabotropic glutamate receptors and calcium signalling in dendrites of hippocampal CA1 neurones. Neuropharmacology 32:1229–1237.

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533–536.

Friel DD, Tsien RW (1992) A caffeine- and ryanodine-sensitive Ca^{2+} store in bullfrog sympathetic neurones modulates effects of Ca^{2+} entry on $[Ca^{2+}]_i$. J Physiol (Lond) 450:217–246.

Garaschuk O, Yaari Y, Konnerth A (1997) Release and sequestration of calcium by ryanodine-sensitive stores in rat hippocampal neurons. J Physiol (Lond) 502:13–30.

Gasparini F, Lingenhohl K, Stoehr N, Flor PJ, Heinrich M, Vranesic I, Biollaz M, Allgeier H, Heckendorn R, Urwyler S, Varney MA, Johnson EC, Hess SD, Rao SP, Sacaan AI, Santori EM, Velicelebi G, Kuhn R (1999) 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. Neuropharmacology 38:1493–1503.

Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260:3440–3450.

Harvey J, Collingridge GL (1993) Signal transduction pathways involved in the acute potentiation of NMDA responses by 1*S*,3*R*-ACPD in rat hippocampal slices. Br J Pharmacol 109:1085–1090.

Irving AJ, Collingridge GL (1998) A characterisation of muscarinic receptor-mediated Ca^{2+} mobilisation in cultured rat hippocampal neurons. J Physiol (Lond) 511:747–759.

Irving AJ, Collingridge GL, Schofield JG (1992) L-Glutamate and acetylcholine mobilise Ca^{2+} from the same intracellular pool in cerebellar granule cells using transduction mechanisms with different Ca^{2+} sensitivities. Cell Calcium 13:293–301.

Jahr CE, Stevens CF (1987) Glutamate activates multiple single channel conductances in hippocampal neurons. Nature 325:522–525.

Koizumi S, Bootman MD, Bobanovic LK, Schell MJ, Berridge MJ, Lipp P (1999) Characterization of elementary Ca^{2+} release signals in NGF-differentiated PC12 cells and hippocampal neurons. Neuron 22:125–137.

Kovalchuk Y, Eilers J, Lisman J, Konnerth A (2000) NMDA receptor-mediated subthreshold Ca^{2+} signals in spines of hippocampal neurons. J Neurosci 20:1791–1799.

Law GL, Pachter JA, Thastrup OM, Hanley R, Dannies PS (1990) Thapsigargin, but not caffeine, blocks the ability of thyrotropin-releasing hormone to release Ca^{2+} from an intracellular store in GH_4C_1 pituitary cells. Biochem J 267:359–364.

Lüthi A, Gähwiler BH, Gerber U (1994) Potentiation of a metabotropic glutamatergic response following NMDA receptor activation in rat hippocampus. Pflügers Arch 427:197–202.

Malinow R, Otmakhov N, Blum KI, Lisman J (1994) Visualizing hippocampal synaptic function by optical detection of Ca^{2+} entry through the *N*-methyl-D-aspartate channel. Proc Natl Acad Sci USA 91:8170–8174.

Mayer ML, Westbrook GL, Guthrie PB (1984) Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. Nature 309:261–263.

Mayer ML, MacDermott AB, Westbrook GL, Smith SJ, Barker JL (1987) Agonist- and voltage-gated calcium entry in cultured mouse spinal cord neurons under voltage clamp measured using arsenazo III. J Neurosci 7:3230–3244.

Mignen O, Shuttleworth TJ (2000) I(ARC), a novel arachidonate-regulated, noncapacitative Ca^{2+} entry channel. J Biol Chem 275:9114–9119.

Miller LD, Petrozzino JJ, Golarai G, Connor JA (1996) Ca^{2+} release from intracellular stores induced by afferent stimulation of CA3 pyramidal neurons in hippocampal slices. J Neurophysiol 76:554–562.

Missiaen L, De Smedt H, Droogmans G, Casteels R (1992) Ca^{2+} release induced by inositol 1,4,5-trisphosphate is a steady-state phenomenon controlled by luminal Ca^{2+} in permeabilized cells. Nature 357:599–602.

Mogami H, Nakano K, Tepikin AV, Petersen OH (1997) Ca^{2+} flow via tunnels in polarized cells: recharging of apical Ca^{2+} stores by focal Ca^{2+} entry through basal membrane. Cell 88:49–55.

Murphy SN, Miller RJ (1989) Two distinct quisqualate receptors regulate Ca^{2+} homeostasis in hippocampal neurons *in vitro*. Mol Pharmacol 35:671–680.

Nakamura T, Barbara J-G, Nakamura K, Ross WN (1999) Synergistic release of Ca^{2+} from IP_3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. Neuron 24:727–737.

Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462–465.

Parekh AB, Penner R (1997) Store depletion and calcium influx. Physiol Rev 77:901–390.

Perkel DJ, Petrozzino JJ, Nicoll RA, Connor JA (1993) The role of Ca^{2+} entry via synaptically activated NMDA receptors in the induction of long-term potentiation. Neuron 11:817–823.

Petrozzino JJ, Pozzo Miller LD, Connor JA (1995) Micromolar Ca^{2+} transients in dendritic spines of hippocampal pyramidal neurons in brain slice. Neuron 14:1223–1231.

Putney JW (1986) A model for receptor-regulated calcium entry. Cell Calcium 7:1–12.

Regehr WG, Tank DW (1992) Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. J Neurosci 12:4202–4223.

Richmond SA, Irving AJ, Molnár E, McIlhinney RAJ, Michelangeli F,

Henly JM, Collingridge GL (1996) Localisation of the glutamate receptor subunit GluR1 on the surface of living and within cultured hippocampal neurones. Neuroscience 75:69–82.
Schiller J, Schiller Y, Clapham DE (1998) NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation. Nat Neurosci 1:114–118.
Segal M, Manor D (1992) Confocal microscopic imaging of $[Ca^{2+}]_i$ in cultured rat hippocampal neurons following exposure to *N*-methyl-D-aspartate. J Physiol (Lond) 448:655–676.
Shmigol A, Kirischuk S, Kostyuk P, Verkhratsky A (1994) Different properties of caffeine-sensitive Ca^{2+} stores in peripheral and central mammalian neurons. Pflügers Arch 426:174–176.
Shmigol A, Svichar N, Kostyuk P, Verkhratsky A (1996) Gradual caffeine-induced Ca^{2+} release in mouse dorsal root ganglia neurons is controlled by cytoplasmic and luminal Ca^{2+}. Neuroscience 73:1061–1067.
Simpson PB, Russell JT (1997) Role of sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPases in mediating Ca^{2+} waves and local Ca^{2+}-release microdomains in cultured glia. Biochem J 325:239–247.
Sitsapesan R, Williams AJ (1990) Mechanisms of caffeine activation of single Ca^{2+} release channels of sheep cardiac sarcoplasmic reticulum. J Physiol (Lond) 423:425–439.
Stanton PK, Sarvey JM (1984) Blockade of long-term potentiation in rat hippocampal CA1 region by inhibitors of protein synthesis. J Neurosci 4:3080–3088.
Takechi H, Eilers J, Konnerth A (1998) A new class of synaptic response involving calcium release in dendritic spines. Nature 396:757–760.
Usachev YM, Thayer SA (1999) Ca^{2+} influx in resting rat sensory neurons that regulates and is regulated by ryanodine-sensitive Ca^{2+} stores. J Physiol (Lond) 519:115–130.
Verkhratsky A, Petersen OH (1998) Neuronal Ca^{2+} stores. Cell Calcium 24:333–343.
Wilsch VW, Behnisch T, Jäger T, Reymann KG, Balschun D (1998) When are class I metabotropic glutamate receptors necessary for long-term potentiation? J Neurosci 18:6071–6080.
Woodhall G, Gee CE, Robitaille R, Lacaille JC (1999) Membrane potential and intracellular Ca^{2+} oscillations activated by mGluRs in hippocampal stratum oriens/alveus interneurons. J Neurophysiol 81:371–382.
Yeckel MF, Kapur A, Johnston D (1999) Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. Nat Neurosci 2:625–633.
Yuste R, Majewska A, Cash SS, Denk W (1999) Mechanisms of calcium influx into hippocampal spines: heterogeneity among spines, coincidence detection by NMDA receptors, and optical quantal analysis. J Neurosci 19:1976–1987.